X-Ray Microscopic Visualization of Specific Labeling of Adhesive Molecule CD36 and Cytoadherence by *Plasmodium falciparum* Infected Erythrocytes

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Cytoadherence between *Plasmodium falciparum* malarial infected red blood cells and venular endothelium results in sequestration of mature parasites in the microvasculature, a condition that contributes to cerebral malaria, the most frequently fatal complication of malaria. Cytoadherence, a biological phenomenon of critical importance to the survival of *P. falciparum* malarial parasites, is the result of specific interaction between receptors on endothelium, including CD36 [1,2], and adhesive ligands that the parasite inserts into the infected erythrocyte membrane, including the antigenically variant family of molecules known as PfEMP1 [3,4]. CD36 is a member of a family of integral membrane glycoproteins that functions both as a cell adhesion molecule and as a scavenger receptor. It is expressed on several cell types, including platelets, monocytes/macrophages, adipocytes, and microvascular endothelial cells.

We used high resolution soft x-ray microscopy to investigate the interactions between target cells that express CD36, malarial infected red blood cell membranes, and intraerythrocytic parasites. We examined surface and internal structures of intact, hydrated, fixed infected erythrocytes and target cells during *in vitro* cytoadherence. We detected 0.2-3m fibrillar structures projecting from the surface of both melanoma and endothelial cells (Figure 1).

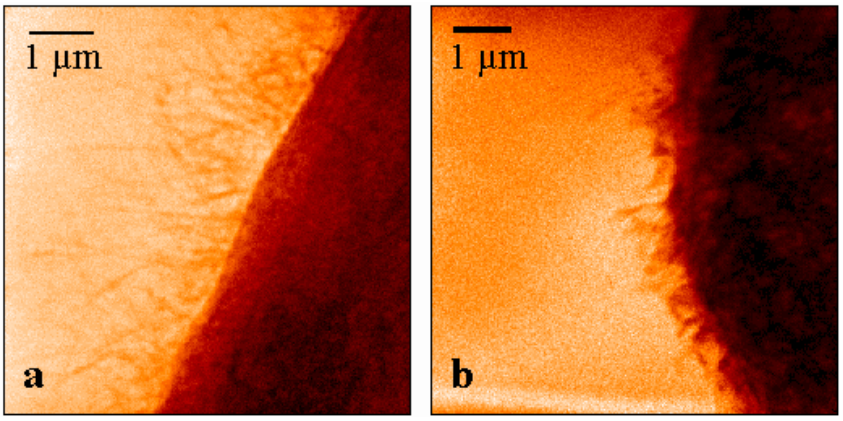

Figure 1. Fibrillar structures projecting from the surface of intact, 1% glutaraldehyde fixed, cultured melanoma cells (a) and endothelial cells (b) are detected by x-ray microscopy.

We investigated the distribution of CD36 on target cells using an immunogold labeling technique that has previously been used in dark-field x-ray microscopy [5]. We examined the orientation of intraerythrocytic parasites in relation to the contact between the erythrocyte membrane and the target cell, and whether membranes of either the erythrocyte or the target cell are detectably altered during cytoadherence. We observed infected erythrocytes in direct contact with the plasma membrane of the target cell, but most interestingly, in some instances the 0.2-3.0 µm fibrillar extensions from the surface of melanoma and endothelial cells appeared to be involved in specific adherence of infected red blood cells. (Fig 2).

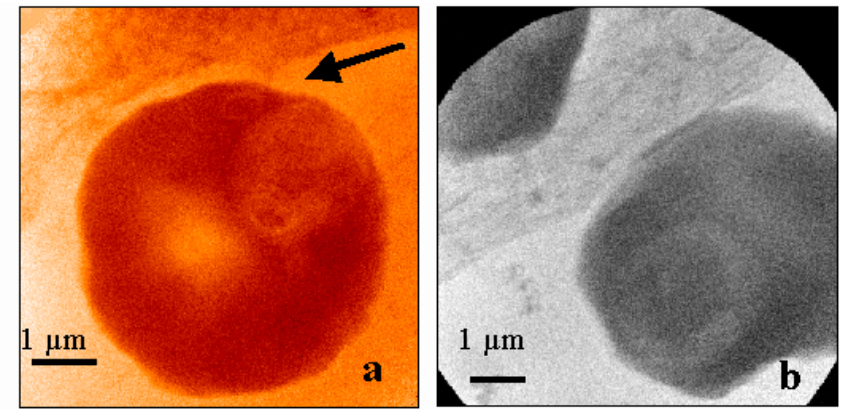

Figure 2. In vitro cytoadherence of *P. falciparum* infected erythrocytes to melanoma or endothelial cells imaged by x-ray microscopy. a) Infected erythrocyte binding to an endothelial cell. Arrow indicates an apparent tether between the cells. Note the vesicular structure adjacent to the intraerythrocytic parasite. b) Infected erythrocyte binding to a melanoma cell.

In our cytoadherence assays, infected erythrocytes tended to bind along the edges of the target cells, so we examined surface distribution of CD36 on melanoma and endothelial cells using monoclonal antibody OKM5 and silver enhanced immunogold labeling. We showed that OKM5 is distributed particularly heavily along the growing edge of melanoma cells (Fig 3). Labeling of CD36 by silver enhanced immunogold beads on endothelial cells was also concentrated along the edges of the cells, but it was very light compared with melanoma cells (data not shown).

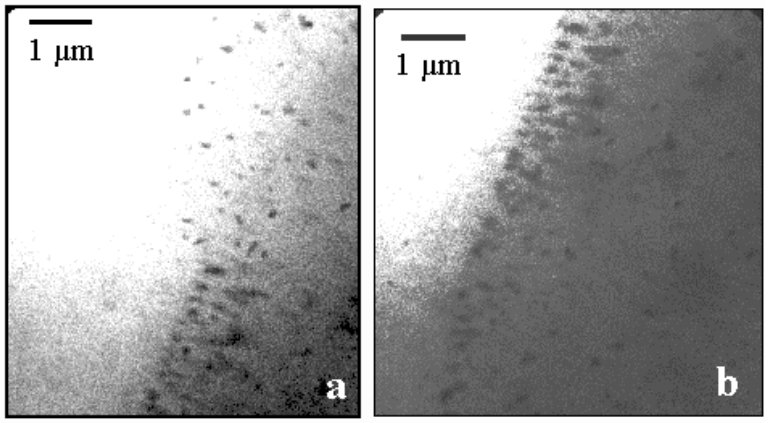

Figure 3. Labeling of CD36 with mAb OKM5 for x-ray microscopic analysis. a,b) Edges of a melanoma cell incubated with OKM5 are heavily decorated with silver enhanced 30nm immunogold beads in these x-ray micrographs. Silver enhancement proceeded under experimentally determined conditions to ensure a high degree of enhancement and low self-nucleation-induced background. For x-rays with 517 eV photon energy (2.4 nm wavelength), the 1/e attenuation length of water, organic material, and silver is about 10m, 0.5m, and 0.05m respectively.

Triton X-100 extracted cells [6] were incubated with OKM5 and a series of images were collected and tiled. Distribution of CD36 throughout the extracted cell can be visualized (Fig 4).

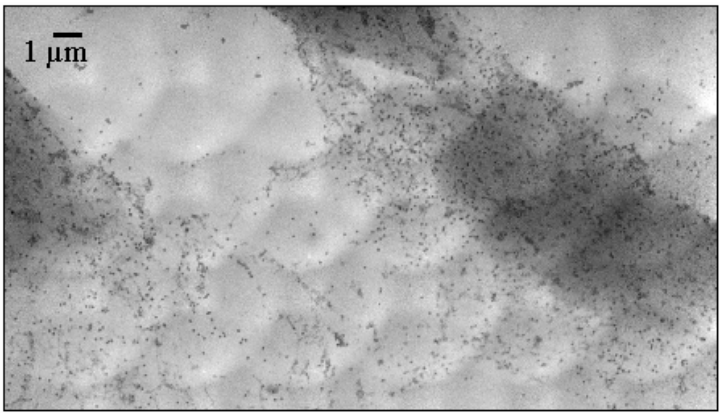

Figure 4. Tiled x-ray image of a Triton X-100 extracted C32 melanoma cell. Extracted cell was incubated with OKM5, followed by immunogold beads enhanced with silver.

Our work provides the first x-ray microscopic images of cytoadherent infected red cells and their interactions with the plasma membrane and surface structures of cells that express CD36. We have

demonstrated that parasites may be oriented in various positions within the red cell cytoplasm during cytoadherence, and that fibrillar structures on the surface of the target cells may be involved in cell-cell adherence. Except for an occasional tether (Fig 2a), changes coincident with cytoadherence were not consistently detected in either the parasite, the red cell membrane, or the target cells. Most alterations of red blood cells, particularly membrane alterations that occur coincident with parasitization and specifically in association with cytoadherence, occur at the biochemical and molecular levels and cannot be detected by microscopy [7,8]. Likewise, potential changes in the membranes of target cells occur at the molecular level [9].

The interaction between *P. falciparum* infected erythrocytes and cells that express CD36, such as venular endothelium in the microvasculature or cultured melanoma cells or endothelial cells, has been studied extensively because *in vivo* it is both critical for survival of the parasite, and potentially fatal for the infected individual. Disruption of the association between infected erythrocytes and the host's venular endothelium is considered a promising vaccine strategy [10]. An eventual understanding of the complex biological interactions and mechanisms which result in cytoadherence and sequestration of malarial infected red cells will contribute to prevention and/or reversal of adherence associated with cerebral malaria.

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